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Effects of willow hybridisation and simulated browsing on the development and survival of the leaf beetle *Phratora vitellinae*

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Abstract

Background: Interspecific hybridisation is common between many plant species and causes rapid changes in a variety of plant characters. This may pose problems for herbivores because changes in recognition characters may be poorly correlated with changes in quality characters. Many studies have examined different systems of hybrids and herbivores in attempts to understand the role of hybridisation in the evolution of plant resistance. The results from different systems are variable. Studies of hybrids between *Salix caprea* (L., Salicaceae) and *S. repens* show that they are intermediate between the two parental species in most resistence characters. However, a plants herbivore resistence depends also on its biotic and abiotic environment. Important biotic factors that may influence plant growth and plant chemistry include the interactions between different herbivores that occur through their exploitation of common host plants. Although the effects on plants of previous herbivory are likely to be strongly affected by environmental conditions, they are also species-specific. Damage may therefore have different effects on hybrids than on their parental species, and this could influence the performance of herbivores on pure and hybrid species of plants. To evaluate the effects of hybridisation on insect performance, the development and survival rates of *Phratora vitellinae* (L. 1758, Coleoptera: Chrysomelidae) larvae on pure *S. repens*, pure *S. caprea* and F1 hybrids of the two species was monitored. Further, to examine the effect of herbivorous mammals on the performance of the larvae, plants were damaged to simulate winter foraging by voles or spring leaf stripping by moose.

Results: The results show that development rates were highest on *S. repens* and equally low on *S. caprea* and the F1 hybrid. In addition, development of the plants treated to simulate mammalian herbivore damage was slower than that of corresponding controls.

Conclusions: The results of this experiment suggest that *P. vitellinae* has a higher development rate, and thus probably higher performance, on species with high concentrations of phenolic glucosides. Therefore, it would be of adaptive benefit for *P. vitellinae* females to have an ovipositional preference for *S. repens*, compared to *S. caprea* and intermediate preference for F1 hybrids. The faster development observed on *S. repens* supports the hypothesis that *P. vitellinae* obtains additional adaptive benefits from phenolic glucosides beyond protection against predators. Therefore, it is important to consider further factors, such as damage caused by other herbivores, when studying this hybrid complex.

Background

To understand the distribution of herbivore populations on different plants we need to consider both host selection and offspring performance on potential hosts [1]. The more similar plants are in recognition characters, and the more variable they are in quality, the more important it is for a herbivore to make appropriate host choices [2–4]. Herbivores' foraging and oviposition decisions are especially challenging when host plant species hybridise with non-host species. It may thus be instructive, especially from an evolutionary perspective, to consider not only distribution and host selection on hybrids but also offspring performance on hybrids, pure hosts and non-hosts that are expected to differ in quality [5].

Interspecific hybridisation is common between many plant species [6] and causes rapid changes in a variety of plant characters [7–10]. This may pose problems for some herbivores because changes in recognition characters may be poorly correlated with changes in quality characters. Many studies have examined different systems of hybrids and herbivores in attempts to understand the role of hybridisation in the evolution of plant resistance [7,11,12]. However, fewer studies have considered the performance of herbivore offspring on hybrid plants [1,13–16].

The amount of resources allocated by a plant to herbivore defence depends on its biotic and abiotic environment, as well as its genotype. Important biotic factors that may influence plant growth and plant chemistry include the interactions between different herbivores that occur through their exploitation of common host plants [17–21]. Although the effects on plants of previous herbivory are likely to be strongly affected by environmental conditions, such as nutrient and water availability, they are also species-specific [18]. Damage may therefore have different effects on hybrids than on their parental species, and this could influence the performance of herbivores on pure and hybrid species of plants.

Willows are extensively used by many different herbivores [22], and willow species are known to hybridise frequently with other willows [23]. However, they can be divided into two different groups based on their leaf chemistry: one with high levels of phenolic glucosides and low levels of condensed tannins and one in which this relationship is reversed [24,25]. Both phenolic glucosides and condensed tannins are secondary chemicals that are known to affect herbivores [22,25,26]. Several different kinds of leaf beetle utilise willows as host species, some of which are adapted to live on willows that are low in phenolic glucosides, while others are adapted to species with high concentrations of these compounds [22,27,25]. *Phratora vitellinae* (L. 1758; Coleoptera: CHRYSOMELIDAE Lat. 1802; insect nomenclature from [28]) is a leaf beetle that is oligophagous on willow species with high concentrations of phenolic glucosides [29,22,25,27]. In the area where the experiments were performed *P. vitellinae* is most often observed on S. myrsinifolia (L.) [30].

In the study reported here I examined the development and foraging of *P. vitellinae* larvae on pure *Salix repens*, pure *S. caprea*, and Fl hybrids between them. From previous studies it is known that *S. repens* has high concentrations of phenolic glucosides, while *S. caprea* has at most low concentrations of these compounds [24,31], and the hybrids are usually intermediate between the parental species in this respect [31]. To study the effect of other herbivores on insect herbivores I also simulated winter foraging by voles and leaf stripping by mammalian herbivores (moose). More specifically, I addressed the following questions:

1. Do the survival and developmental rates of *P. vitellinae* larvae growing on *S. repens*, *S. caprea* and Fl hybrids differ?

2. Does the amount of leaf area consumed by *P. vitellinae* larvae differ between *S. repens*, *S. caprea* and Fl hybrids?

3. Does simulated herbivory influence the survival and development of *P. vitellinae*?

4. Does simulated herbivory influence the amount of leaf area consumed by *P. vitellinae* larvae?

Results

There were significant effects of both plant category and treatment on development, but not on survival (Figures, 1 and 2 and Tables 1 and 2). There was a higher probability for a surviving individual to develop to adult or pupa stage before the end of the experiment on *S. repens* than on *S. caprea* ($p = 0.04$), but no significant difference between the Fl hybrid and *S. caprea* in this respect ($p = 0.86$). There was also a slightly lower probability for individuals to reach pupa or adult stage on plants treated to simulate leaf stripping by a browser than on control plants or plants treated to simulate winter foraging by voles or hares ($p = 0.07$ for control and $p = 0.05$ for simulated vole damage).

There were no significant differences in consumed leaf area between plant categories (ANOVA $F = 0.51$, $p = 0.60$; Figure 3) or between treatments (ANOVA $F = 1.77$, $p = 0.18$; Figure 4), and no significant interaction between treatment and plant category.

Discussion

Development of *P. vitellinae* larvae is equally slow on Fl hybrid plants as on the non-salicylate parental species, *S.*

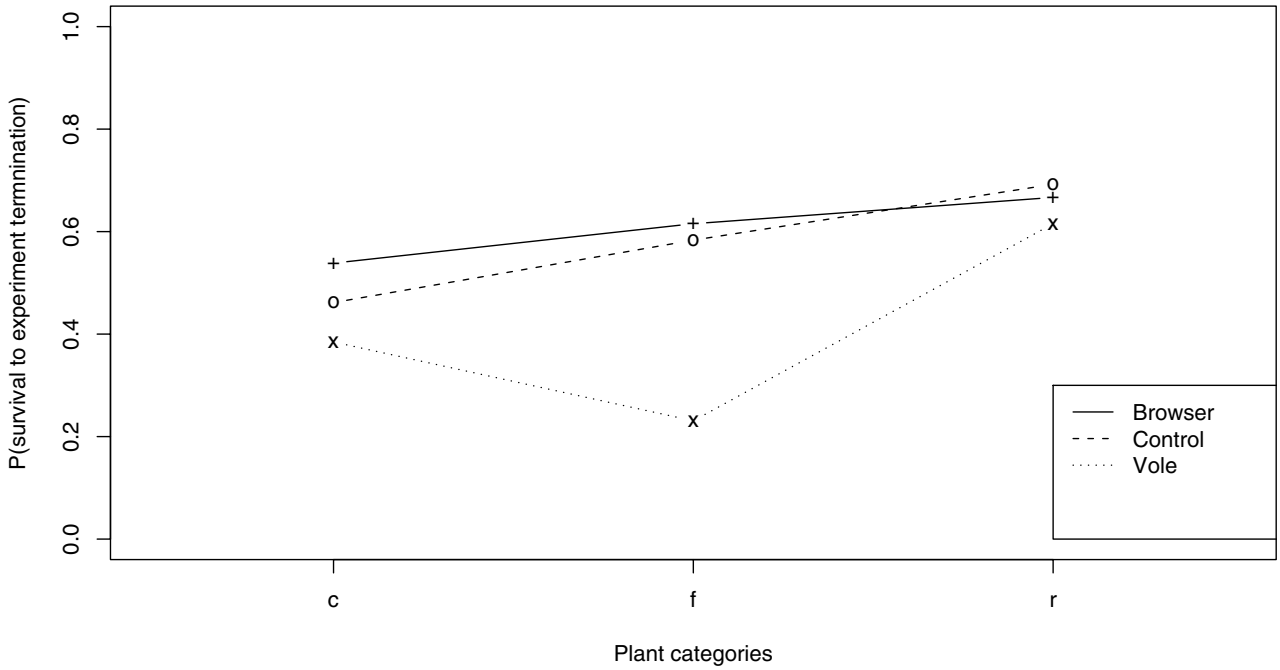

Figure 1
Larvae survival Probability (y-axes) of a surviving *P. vitellinae* larva reaching adult or pupal stage by the end of the experiment for *S. caprea* (c), Fl hybrid (f) and *S. repens* (r), and the three treatments, browser – simulated herbivore damage through leaf stripping and removal of apical bud, to simulate the foraging of a browser, for example moose, control – no damage and vole = simulated herbivore damage through cutting shoots to simulate winter foraging of voles.

caprea, but significantly faster on pure *S. repens*. Since the time spent as larvae affects several factors that, in turn, influence the risk of mortality (e.g. predation and the scope for choosing over-wintering sites), I believe that it is safe to argue that rapid development from egg to adult is indicative of high performance. The results are consistent with reports that the insect's preference for salicylic plants is of adaptive benefit [25,27]. It has been suggested that the preference of *P. vitellinae* for phenolic glucosides may be related to its ability to synthesise salicylaldehydes from phenolic glucosides for its own defence, although several studies have shown that other factors may be involved. *P. vitellinae* larvae convert salicyl glucosides from the plants they feed on into salicylaldehydes [25], which are excreted onto their backs when they are disturbed and are believed to act as deterrents against generalist predators [32,25]. However, it has also been shown that even in the absence of predators, survival and development rates tend to be higher when the insects feed on plants with high amounts of phenolic glucosides [25,27]. It has been suggested that this may be due to *P. vitellinae* metabolising the sugar from the sequestered phenolic glucosides [25]. In the present study I found no difference in survival between plant categories, but significant differences in the development time to the pupal stage.

In a previous study Fl hybrids proved to be intermediate between parental species in phenolic glucoside concentration [31]. If the phenolic glucoside concentration really does influence larval performance, more than any other character, the results indicate that *P. vitellinae* may require leaves with more than a specific threshold concentration to provide optimal nutrition. It is also interesting to note that although performance was greater on *S. repens*, a large proportion of the larvae on *S. caprea* and the Fl hybrid were still developing when I terminated the experiment. This shows that under enemy-free conditions, phenolic glucosides are not essential dietary components for *P. vitellinae*.

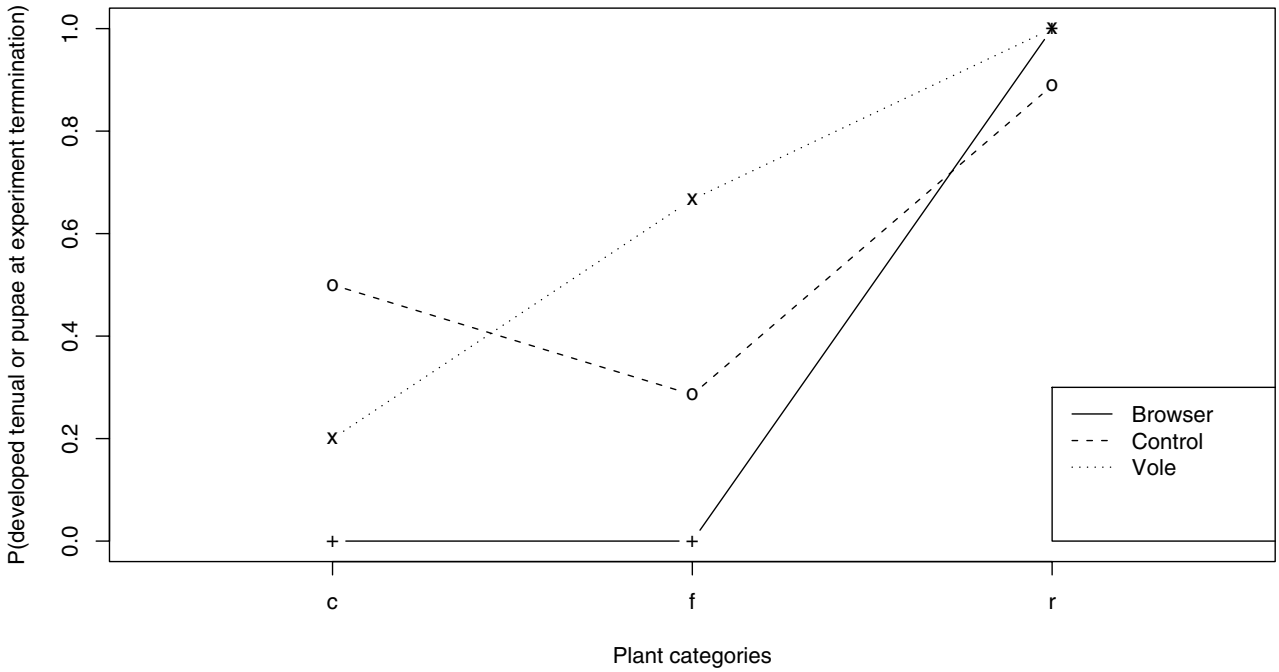

Figure 2
Larvae development Probability (*y*-axes) of a *P. vitellinae* larva surviving from the start to the end of the experiment. For further explanation and abbreviations see Figure 1.

Table 1: Sequential analyses of deviance for the logistic regression analyses of development. The data show there were significant effects of plant category and treatment, and a close to significant interaction between plant category and treatment.

Factor	D.f.	Deviance	Residual D.f.	Residual Deviance	$P(>\|\chi^2)$
Model			60	84.42	
Plant category	2	37.88	58	46.54	<0.01
Treatment	2	6.05	56	40.49	0.049
Interaction	4	8.69	52	31.80	0.07

Table 2: Sequential analyses of deviance for the logistic regression analyses of survival. The data show there were no significant effects of plant category, treatment or the interaction between plant category and treatment.

Factor	D.f.	Deviance	Residual D.f.	Residual Deviance	$P(>\|\chi^2)$
Model			113	157.48	
Plant category	2	3.55	111	153.92	0.17
Treatment	2	3.24	109	150.68	0.20
Interaction	4	1.70	105	148.98	0.79

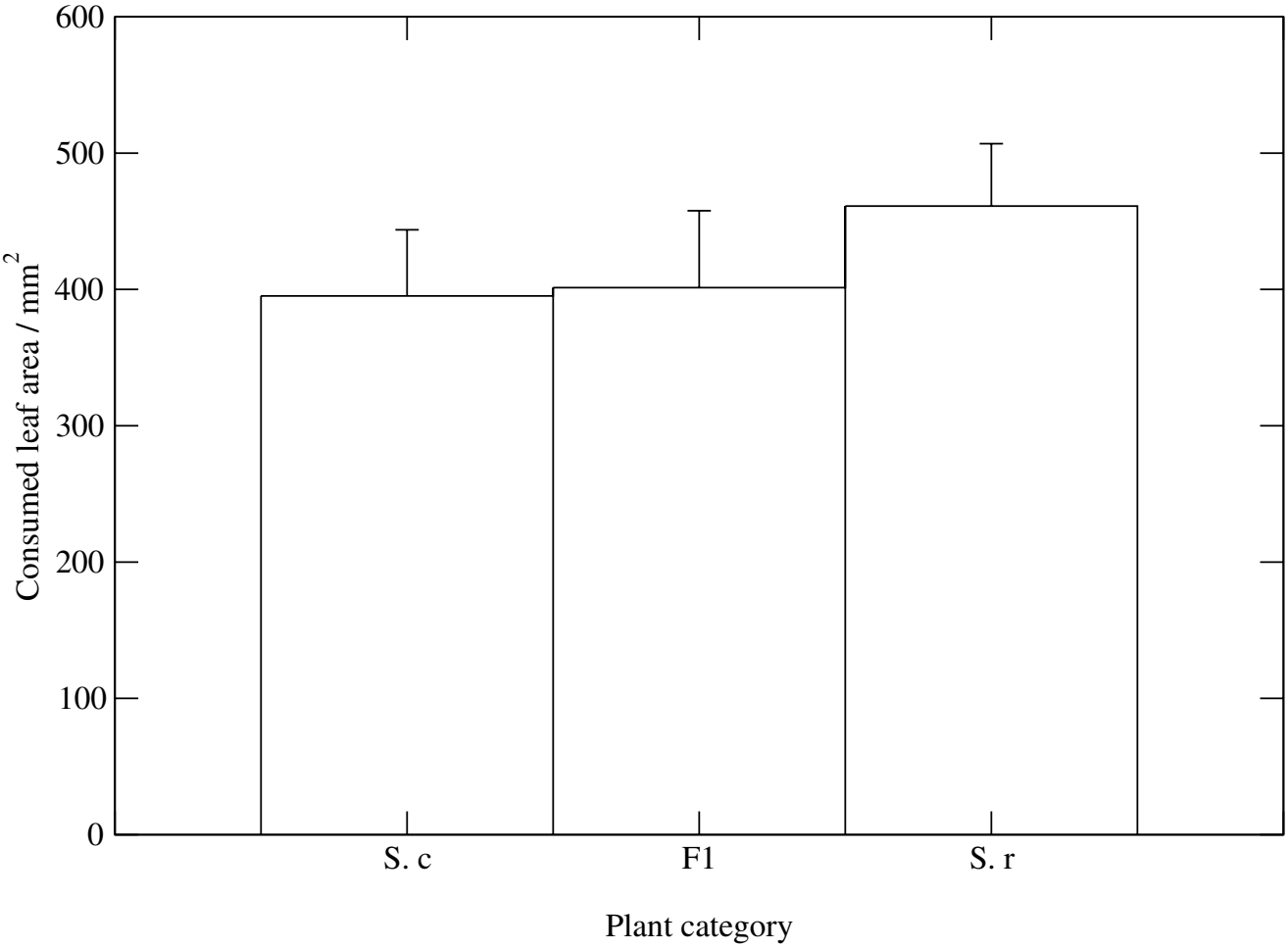

Figure 3
Differences in larval foraging between plant categories Mean leaf area consumed by *P. vitellinae* larvae for *S. caprea* (S. c), Fl hybrids (Fl) and *S. repens* (S. r). Bars represent standard errors of means.

It has been suggested that *P. vitellinae* displays a compensatory feeding behaviour, eating more when forced to feed on leaves with low levels of phenolic glucosides [27]. Nevertheless, although there were differences in developmental stage between plant categories when the experiments were terminated, there were no differences in the leaf area consumed. This indicates that the larvae may need to forage less on *S. repens* than on the Fl hybrid or *S. caprea* to reach the same developmental stage. However, although phenolic glucosides are clearly important to these beetles, other plant characters that may differ between pure and hybrid individuals may also be important [25]. For instance, other studies of *P. vitellinae* have shown that leaf trichomes have a clear effect on both larval performance and female host selection [33,34]. However, all plant types tested in this study, both hybrids and the pure species, have hairs on their leaf surfaces, irrespective of whether their phenolic glucoside concentrations are low or high, so the differences observed in this case are unlikely to be due to the presence or absence of trichomes. Herbivore development is not influenced solely by secondary metabolites in the food plants, it is also clearly dependent on the amount of primary nutrients they contain, especially nitrogen and carbohydrates [35,36]. Even though the plants in the present study were treated equally, they may have differed in primary nutrient contents, and these differences may also have affected the development of *P. vitellinae* larvae.

For the two plant categories on which larval development was reduced, *S. caprea* and the Fl hybrid, the simulated leaf stripping, which included removal of the leafs on the

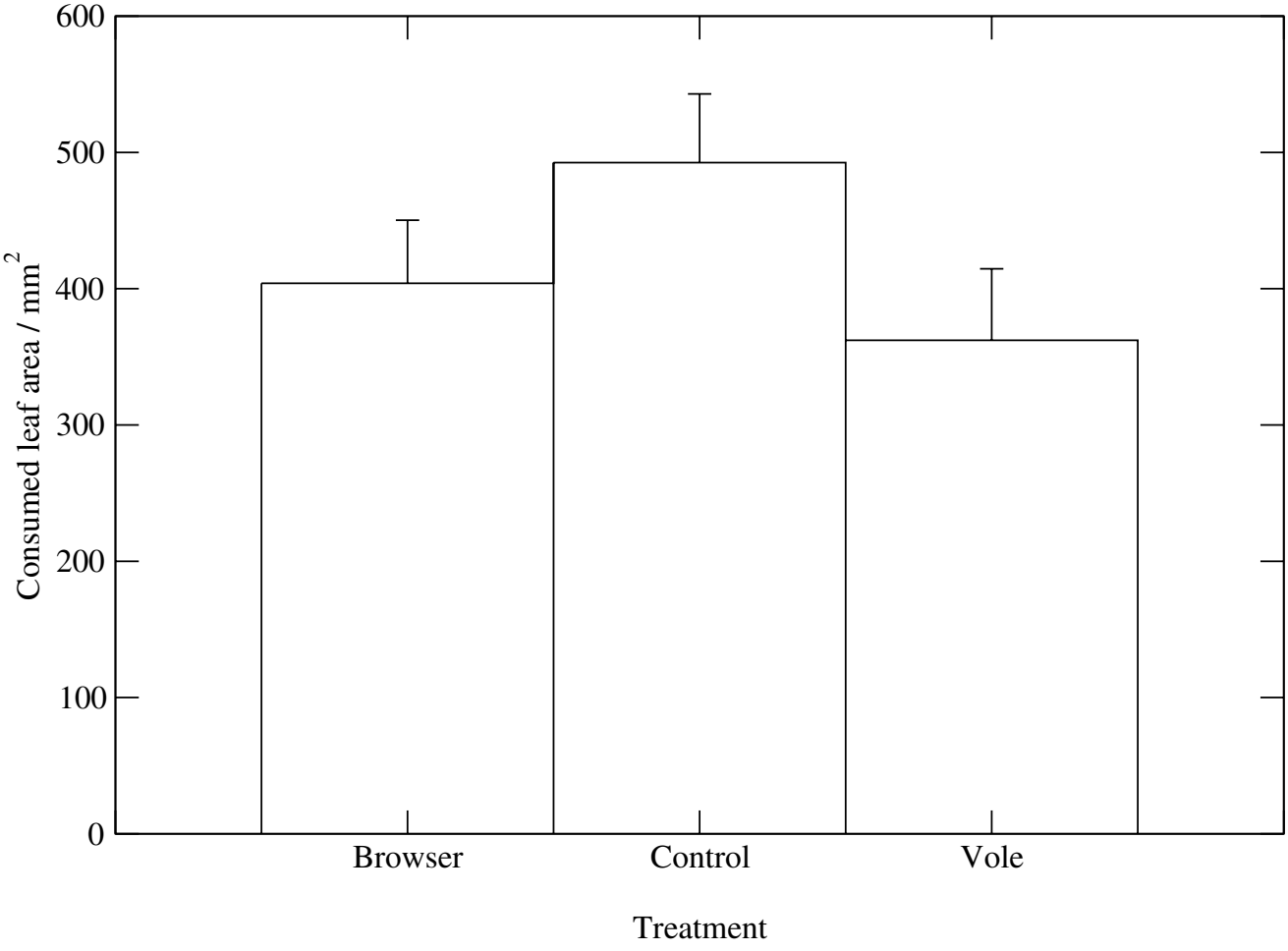

Figure 4
Differences in larval foraging between treatments Mean leaf area consumed by *P. vitellinae* larvae for each treatment.

Table 3: Plant crosses, and the number of individuals used from each cross. Maternal plants are listed in the row headings and the paternal plants in the column headings. *S. caprea* parents are denoted by C, and *S. repens* parents by R.

	C524	C621	C721	R225	R223	R422	421
C512	33						
C513		6					
C514						35	13
C611			18				9
R11					31		
R211				26			

terminal shoot and the apical bud, reduced development rates even further. Hence, the plant categories might differ in the way their response to damage affects *P. vitellinae* larvae. Such differences would not be surprising since the plants are morphologically very different [37,23]. *S. caprea* adopts an upright tree habit, and thus has fairly strong apical dominance, while *S. repens* has a creeping growth pattern and weak apical dominance [37,23]. The hybrid plants are commonly intermediate in growth pattern between the parental species, although there is also great variation in morphology, and individuals may be more similar to either one of the parental species. The secondary chemistry may be more affected by damage in plants that have strong apical dominance, than in plants with weak apical dominance [18]. This is because source-sink relationships, and thus intra-plant regulatory processes, are affected when apical dominance changes. If the plant has weak apical dominance, damage to a shoot will not change the source-sink structure as much as if the plant has strong apical dominance [18].

Conclusions

This study shows that there would be adaptive value for *P. vitellinae* females to show oviposition preference for *S. repens*, compared to *S. caprea* and Fl hybrids between *S. caprea* and *S. repens*, under the conditions of these experiments. The faster development observed on *S. repens* supports the hypothesis that *P. vitellinae* obtains additional adaptive benefits from phenolic glucosides, as shown by Rank et al. [27], beyond protection for the larvae against predators. It may also be important to consider damage caused by other agents, for example other herbivores, when studying this hybrid complex, as there are indications that plant responses to damage differ between *S. repens*, *S. caprea* and the Fl hybrid.

Methods

Plant material

Pure and hybrid plants were grown from seeds collected from naturally growing parents hand-pollinated with pollen from either individuals of the same species or from the other parental species used in this study. Individuals with typical characters for their respective species [37], indicating genetic purity, were selected for use as parents. For details about the hand pollination methods see [38] and Table 3. Seeds were planted in pots (0.25 1) in a greenhouse and grown under 12 h photoperiods from mid-April to October and natural light conditions during the winter (temperature ≤ 15°C throughout). Plants were supplied with water when needed, about every third day, and fertilized once a week with liquid fertilizer ("SuperbaS"; NPK 6.5:1.0:4.7) after being placed in a factorial split-plot design with nine plants in each of 13 blocks. Each block consisted of three sub-blocks (one sub-block for each treatment) with one plant from each plant category (S. caprea, S. repens and Fl hybrid) randomly assigned to positions in the sub-block. During late winter (early March) the main shoot on each of one sub-block of plants was cut 10 cm above ground before leaf emergence, to simulate winter foraging by voles or hares. At this stage the plants were approaching one year of age and they were still dormant. Later, during early spring after leaf emergence (mid-May), the uppermost 15 cm of the main shoot of each plant in a different sub-block was defoliated and the apical bud was removed, to simulate leaf stripping by a browser, for example moose. Plants in the last sub-block were left untreated as controls. Treatments were randomly distributed among the sub blocks within each block. Due to differences in morphology between the plants it was not possible to remove the same amount of plant material, and still leave the plants in similar conditions, in the treatment that simulated vole winter foraging. On average 0.47 g dry weight (95% CI ± 0.07) was removed from S. caprea, 0.85 g dry weight (95% CI ± 0.17) from Fl hybrids, and 0.87 g dry weight (95% CI ± 0.16) from S. repens. There were no significant differences in the amount of material removed from the three types of plants when simulating moose leaf stripping, average amounts being 0.13 g dry weight (95% CI ± 0.06) for S. caprea, 0.17 g dry weight (95% CI ± 0.04) for Fl hybrids, and 0.13 g dry weight for S. repens (95% CI ± 0.03).

Insects

First instar *P. vitellinae* larvae were collected on July 1 in the field from S. myrsinifolia (L.). On each experimental plant a larva was enclosed on a randomly chosen shoot, with sufficient leaves to support its growth. Larvae for each block were taken from the same wild plant, and thus probably originated from the same clutch. The larvae were left to forage and develop on the plant for 46 days (until August 16), when the first beetles hatched from their pupae. At this point I measured survival, the developmental stage the larvae had attained and the area of consumed leaf. When the experiment was terminated no larvae had eaten all of the enclosed leaves.

Statistical methods

I analysed whether there were any differences between plant categories and treatments in the probability of a larva surviving until the termination of the experiment. Among the surviving individuals I analysed the influence of plant category and simulated herbivory treatment on the probability of a *P. vitellinae* individual reaching pupal or adult stage at the end of the experiment. For both analyses I used logistic regression with plant category, treatment and the interaction between them as factors in the model [39,40]. To evaluate differences in the consumed leaf area I used two-factorial analyses of variance with plant categories and treatments as independent factors. For all statistical tests I used R version 1.2 [41].

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References

1. Orians C, Huang C, Wild A, Dorfman K, Zee P, Dao M and Fritz R: **Willow hybridization differentially affects preference and performance of herbivourous beetles** *Ent Exp Appl* 1997, **83:**285-294.
2. Fox C and Lalonde R: **Host confusion and the evolution of insect diet breadths** *Oikos* 1993, **67:**577-581.
3. Janz N and Nylin S: **The role of female search behaviour in determining host plant range in plant feeding insects: a test of the information processing hypothesis** *Proc R Soc Lond B Biol Sci* 1997, **264:**701-707.
4. Bernays E: **The Value of Being a Resource Specialist: Behavioral Support for a Neural Hypothesis** *Am Nat* 1998, **151:**451-464.
5. Hallgren P: **Ecological consequences of plant hybridization in willows: Inheritance patterns of secondary compounds and herbivore foraging behaviour** *Ph.D. thesis, Swedish University of Agricultural Sciences, Umeå* 2002[http://www.abc.se/~m11032/].
6. Arnold M, Bulger M, Burke J, Hempel A and Williams J: **Natural hybridization: How low can it go and still be important?** *Ecology* 1999, **80:**371-381.
7. Strauss S: **Levels of herbivory and parasitism in host hybrid zones** *Trends Ecol Evol* 1994, **9:**209-214.
8. Moulia C: **Parasitism of plant and animal hybrids: are facts and fates the same?** *Ecology* 1999, **80:**392-406.
9. Fritz R, Moulia C and Newcombe G: **Resistance of hybrid plants and animals to herbivores, pathogenes, and parasites** *Annu Rev Ecol Syst* 1999, **30:**565-591.
10. Orians C: **The effects of hybridization in plants on secondary chemistry: implications for the ecology and evolution of plant-herbivore interactions** *Am J Bot* 2000, **87:**1749-1756.
11. Fritz RS: **Resistance of hybrid plants to herbivores: genes, environment, or both?** *Ecology* 1999, **80:**382-391.
12. Whitham T, Martinsen G, Floate K, Dungey H, Potts B and Keim P: **Plant hybrid zones affect biodiversity: tools for a genetic-based understanding of community structure** *Ecology* 1999, **80:**416-428.
13. Hanhimäki S, Senn J and Haukioja E: **Performance of insect herbivores on hybridizing trees – the case of the sub-arctic birches** *J Anim Ecol* 1994, **63:**163-175.
14. Gange A: **Aphid performance in an alder (A1nus** .
15. Messina F, Richards J and McArthur E: **Variable responses of insects to hybrid versus parental sagebrush in common gardens** *Oecologia* 1996, **107:**513-521.
16. Simens DH, Ralston BE and Johnson CD: **Alternative seed defense-mechanisms in a palo verde (*Fabaceae*) hybrid zone – effects on Bruchnid beetle abundance** *Ecol Entom* 1994, **19:**381-390.
17. Danell K and Huss-Danell K: **Feeding by insects and hares on birches earlier affected by moose browsing** *Oikos* 1985, **44:**75-81.
18. Honkanen T and Haukioja E: **Intra-plant regulation of growth and plant-herbivore interactions** *Ecoscience* 1998, **5:**470-479.
19. Hjältén J: **Willow response to pruning: The effect on plant growth, survival and suceptability to leaf gallers** *Ecoscience* 1999, **6:**62-67.
20. Price P, Roininen H and Ohgushi T: **Comparative plant-herbivore interactions involving willows and three gall-inducing sawfly species in the genous *Pontania* (Hymenoptera: Tenthredinidae)** *Ecoscience* 1999, **6:**41-50.
21. Lehtilä K, Haukioja E, Kaitaniemi P and Laine K: **Allocation of resources within mountain birch canopy after simulated winter browsing** *Oikos* 2000, **90:**160-170.
22. Tahvanainen J, Julkunen-Tiitto R and Kettunen J: **Phenolic glycosides govern the food selection pattern of willow feeding leaf beetles** *Oecologia* 1985, **67:**52-56.
23. Skvortsov AK: **Willows of Russia and adjacent countries. Taxonomical and geographic revision** *Faculty of Mathematics and Natural Sciences Report Series Volume 39. University of Joensuu*; 1999.
24. Julkunen-Tiitto R: **Distribution of certain phenolics in *Salix* species (Salicaceae)** *Ph.D. thesis, University of Joensuu, Joensuu* 1989.
25. Pasteels J and Rowell-Rahier M: **The chemical ecology of herbivory on willows** *Proceedings of the Royal Society of Edinburgh section Biological sciences* 1992, **98:**63-73.
26. Ayres M, Clausen T, MacLean S, Redman A and Reichardt P: **Diversity of structure and antiherbivore activity in condensed tannins** *Ecology* 1997, **78:**1696-1712.
27. Rank N, Köpf A, Julkunen-Titto R and Tahvanainen J: **Host preference and larvae performance of the salycylate-using leaf beetle *Phratora vitellinae*** *Ecology* 1998, **79:**618-631.
28. Gustavsson B: **Catalogus Coleopterorum Sueciae** 2002[http://www.nrm.se/en/catalogus.html.se].
29. Pasteels J, Rowell-Rahier M, Braekman J and Daloze D: **Chemical defences in leaf beetles and theire larvae: the ecological, evolutionary and taxonomic significance** *Biochem Syst Ecol* 1984, **12:**395-406.
30. Sipura M: **Herbivory on willows: abiotic constraints and thophic interactions** *Ph.D. thesis, University of Joensuu* 2000.
31. Hallgren P, Ikonen A, Hjältén J and Roininen H: **Inherritance patterns of phenolics in FI, F2 and back-cross hybrids of willows: implications for herbivore responses to hybrid plants** *J Chem Ecol* 2003, **29:**1119-1134.
32. Denno R, Larson S and Olmstead K: **Role of enemy-free space and plant quality in host-plant selection by willow beetles** *Ecology* 1990, **71:**124-137.
33. Rowell-Rahier M and Pasteels J: **The significance of salicin for a *Salix*-feeder, *Phratora* (*Phyllodecta*) *vitellinae*** *In: Procedings 5th international Symposium Insect-Plant Relationsships, Wagenin-gen, Pudoc* 1982:73-79.
34. Soetens P, Rowell-Rahier M and Pasteels J: **Influence of phenolglucosides and trichome density on the distribution of insect herbivores on willows** *Ent Exp Appl* 1991, **59:**175-187.
35. Schoonhoven L, Jermy T and van Loon J: **Insect-plant biology: From physiology to evolution** *London, Chapman & Hall* 1998.
36. Raubenheimer D and Simpson SJ: **Integrating nutrition: a geometrical approach** *Ent Exp Appl* 1999, **91:**67-82.
37. Lid J and Tande Lid D: **Flora of Norway and Sweden (in Norwegian)** *Det Norska Samlaget* 1994.
38. Hjältén J: **Willow hybrids and herbivory: a test of hypotheses of phytophage response to hybrid plants using the generalist leaf-feeder *Lochmea caprea* (Chrysomelidae)** *Oecologia* 1997, **109:**571-574.
39. Selvin S: **Modern applied biostatistical methods using S-plus** *Monographs in epidemiology and bio statistics Volume 28. Oxford University Press*; 1998.
40. Venables W and Ripley B: **Modern applied statistics with S-Plus** *New York, Springer-Verlag* 1999.
41. Venables W and Smith D: **An Introduction to R** *The R-development core team* 2002[http://www.r-project.org/].